WS11.5 Reversible proline hydroxylation in the guanylate cyclase SadC regulates alginate export in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa up-regulates production of the exopolysaccharide alginate under oxygen limitation leading to biofilm formation and pathogen persistence in air-ways of patients with cystic fibrosis (CF). Our previous results demonstrated that in *P. aeruginosa* the three component system (OraB/OraA/SadC) controls alginate production and mucoidy via production of the second messenger cyclic bis-(3′-5′)-diguanylate monophosphate (c-di-GMP). Here we show that in the presence of oxygen purified OraA inhibits c-di-GMP production of the purified cytosolic fragment of the diguanylate cyclase SadC, while purified OraB re-activates SadC to produce c-di-GMP. To elucidate the reaction mechanism, we synthesized a 20 amino acid peptide, containing the structural domain of SadC, GGEEF. Incubation of the peptide with the hydroxylase OraA resulted in hydroxylation of a proline residue (HO-P), adjacent to GGEEF. Incubation of the HO-P containing peptide with OraB resulted in dehydrata-tion of the proline residue. Incubation of a peptide carrying a dehydroproline with OraA resulted again in HO-P formation. This novel post-translational regulatory mechanism links oxygen sensing with c-di-GMP-coupled signal-transduction and exopolysaccharide production in *P. aeruginosa*.

WS11.6 Environmental *Burkholderia cenocepacia* strains can disrupt epithelial integrity in bronchial epithelial cells *in vitro* and have a more profound effect on ZO-1 in CF cells

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Burkholderia cenocepacia is an important human pathogen in patients with cystic fibrosis (CF). Non-clinical reservoirs may play a role in the acquisition of infections, so it is fundamental to evaluate the pathogenic potential of environmental *B. cenocepacia* isolates. We investigated the interactions of two environmental *B. cenocepacia* strains (Mex1 and MCII-168) with two bronchial epithelial cell lines, 16HBE14o– and CFBE41o–, with a non-CF and a CF phenotype, respectively. The environmental strains showed a significantly lower level of invasion into both cell lines compared with the clinical *B. cenocepacia* LMG16656^T strain. Exposure of polarized CFBE or 16HBE cells to the environmental strains resulted in a significant reduction in transepithelial resistance (TER), comparable to that shown by exposure to the clinical strain. A different mechanism of tight junction (TJ) disruption in CF versus non-CF epithelia was found. In 16HBE cells, the environmental strains resulted in a drop in TER without any apparent effect on TJ proteins such as ZO-1, whereas in CFBE cells, the level and localisation of ZO-1 were dramatically altered by the presence of both the environmental strains, comparable to the effect of LMG16656. This demonstrates although the environmental strains are significantly less invasive than the clinical strain, they have an effect on epithelial integrity comparable to the clinical strain. Finally, the TJ protein ZO-1 appears to be more susceptible to the presence of environmental strains in CF cells than in non-CF cells.

This work was funded by the Italian CF Research Foundation (Grants FFC#7/2006 and FFC#7/2008 and HEA PRTLI Cycle 4 Ireland.

WS11.7 Expression of the NF-κB inhibitor A20 is altered in the cystic fibrosis epithelium

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Objectives: A20, a zinc finger protein inducible in response to LPS, is a critical inhibitor and regulator of NF-κB. A20 inhibits TLR-induced NF-κB signalling at the level of TRAF6, but must form a complex with Ring Finger protein (RNF)11, Itch and TAX1BP1 [1]. We hypothesised that loss or variation of normal A20 function may contribute to uncontrolled inflammation in Cystic Fibrosis epithelium.
Methods: Cell lines (16HBE41o–, CFBE41o–) and nasal epithelial cells (NECs) from patients (F508del homozygous) and age-matched controls were stimulated with LPS (*P. aeruginosa*, Sigma) for 0–24h. Expression and interaction of complex members was assessed by qPCR and immunoprecipitation. The C-terminal domain of A20 was assessed by Western Blot.
Results: A20 induction peaked at 1h in 16HBE14o– but was delayed until 4h in CFBE41o–. A20 expression fell significantly below basal levels by 12h only in CFBE41o– where persistent p65 expression and IL-8 release was observed. These observations were confirmed in primary CF NECs where a significant inverse relationship between A20 and p65 was established. Reduced expression of all complex members was observed in CF cells following stimulation, implying a potential defect in the formation of the complex. A20 interacted with RNF11 and TRAF6 in stimulated 16HBE14o–, but not in CFBE41o–. The C-terminal domain of A20 facilitates A20 interaction with complex members and TRAF6 [2], but CFBE41o– cells poorly expressed the C-terminus.
Conclusions: Overall, we report significant deviations of A20 expression in the CF epithelium that may play a role in chronic and persistent airways inflammation.
Supported by CF Trust (PJ541).

Reference(s)

[1] Shembade *et al.* 2009.
[2] Shembade *et al.* 2010.

WS11.8 Adaptation and survival of *Burkholderia cenocepacia* within the CF lung throughout the course of infection

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Burkholderia cepacia complex, (Bcc) comprises seventeen bacterial species that cause severe respiratory infections in Cystic Fibrosis (CF). These pathogens are clinically challenging as they are highly transmissible and antibiotic resistant. This study investigates the virulence features of 3 *B. cenocepacia* clonal variants isolated from a CF patient over four years.
The potential adaptation of *B. cenocepacia* to lung conditions during chronic infection was examined. The two later isolates opened epithelial tight junctions more rapidly than the initial isolate (2hrs Vs 6hrs), suggesting that during chronic infection these strains adapt to gain access to underlying tissue. Furthermore, the two later isolates were on average more than 4 times more invasive of epithelial cells than the first isolate and induced significantly greater levels of IL-6 and IL-8 from epithelial cells compared to the initial isolate ($P < 0.001$).
The ability of these clonal variants to utilize host proteins as an iron source was investigated. It was found that they can all utilize hemin and ferritin as an iron source in an iron deprived environment. In response to *P. aeruginosa* exoproducts, gene expression of the siderophore ornibactin is significantly up-regulated in all isolates ($P < 0.001$), although their growth is inhibited by 50%, indicating that iron acquisition strategies are important for survival in the lung.
Ongoing studies are aimed at a greater understanding of the potential adaptation of *B. cenocepacia* in the CF lung and the role of iron availability in *B. cenocepacia* pathogenesis.
Funded by Technological Sector Research Programme Strand III.